review paper

SEORious business: structural proteins in sieve tubes and their involvement in sieve element occlusion

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Abstract

The phloem provides a network of sieve tubes for long-distance translocation of photosynthates. For over a century, structural proteins in sieve tubes have presented a conundrum since they presumably increase the hydraulic resistance of the tubes while no potential function other than sieve tube or wound sealing in the case of injury has been suggested. Here we summarize and critically evaluate current speculations regarding the roles of these proteins. Our understanding suffers from the suggestive power of images; what looks like a sieve tube plug on micrographs may not actually impede translocation very much. Recent reports of an involvement of SEOR (sieve element occlusion-related) proteins, a class of P-proteins, in the sealing of injured sieve tubes are inconclusive; various lines of evidence suggest that, in neither intact nor injured plants, are SEORs determinative of translocation stoppage. Similarly, the popular notion that P-proteins serve in the defence against phloem sap-feeding insects is unsupported by empirical facts; it is conceivable that in functional sieve tubes, aphids actually could benefit from inducing a plug. The idea that rising cytosolic Ca²⁺ generally triggers sieve tube blockage by P-proteins appears widely accepted, despite lacking experimental support. Even in forisomes, P-protein assemblages restricted to one single plant family and the only Ca²⁺-responsive P-proteins known, the available evidence does not unequivocally suggest that plug formation is the cause rather than a consequence of translocation stoppage. We conclude that the physiological roles of structural P-proteins remain elusive, and that in vivo studies of their dynamics in continuous sieve tube networks combined with flow velocity measurements will be required to (hopefully) resolve this scientific roadblock.

Key words: Forisome, papilionoid legumes (Fabaceae sensu stricto), phloem transport, P-protein, sieve element occlusion (SEO) protein, sieve element occlusion-related (SEOR) protein, sieve tube slime.

Introduction: struggling with structural sieve tube components

The practical investigation of biochemical and molecular properties of cell components usually starts from an initial isolation and purification. The isolation of a specific structure or substance may be extremely complicated if it is present in low quantities. This is a common problem when working with phloem components. Sieve elements, companion cells, and phloem parenchyma are very different cell types, but form a functional unit of structurally and functionally interconnected elements (Esau, 1969; Evert, 1982; Knoblauch and Peters, 2010). The separation of one cell type from the others is practically impossible. In addition, the phloem forms a network, embedded in other tissues. The phloem contributes <1% of the plant body in most species, which complicates isolation and purification even more. Historically, biochemical and molecular investigations into phloem composition were mostly restricted to soluble substances that could be found in phloem sap exudates (Atkins et al., 2010).
It is no surprise that the first thorough biochemical studies of phloem components were conducted on cucurbits (Cucurbitaceae) which exude phloem sap over prolonged periods when treated properly, enabling the collection of milliliter volumes of sap (Crafts, 1932). Components such as the phloem filament protein (PP1) and phloem lectin (PP2) were isolated in large quantities, allowing thorough biochemical analysis (Lin et al., 2009). The phloem of many cucurbits is unusual not only because of the bicollateral vascular bundles in which two sets of sieve tubes are located externally and internally of the xylem, but also because of an extrafascicular sieve tube network that is scattered throughout the non-vascular parenchyma (Fischer, 1884; Crafts, 1932). Unfortunately, most of the exudate that can be easily collected in cucurbits does not seem to originate from the phloem at all (Zhang et al., 2012). Furthermore, it is not really clear how much exudate is contributed from the extrafascicular phloem whose contents and function differ significantly from those of the vascular phloem (Zhang et al., 2010; Gaupels et al., 2012; Zhang et al., 2012). This makes comparisons with non-cucurbit species difficult, and suggests that results obtained with cucurbits should not be rashly generalized (Turgeon and Oparka, 2010; Slewinski et al., 2013).

Exudates—although usually in lower quantities compared with cucurbits—can also be collected from other plant species, and a variety of soluble substances including proteins, RNAs, amino acids, and sugars have been detected (Marentes and Grusak, 1998; Schobert et al., 2000; Lough and Lucas, 2006). Structural components, however, are usually absent from exudates due to their size and insolubility. Until recently it was believed that these structural components comprise endoplasmic reticulum (ER), mitochondria, sieve element plastids, and phloem-specific proteins (P-proteins; for a review, see Knoblauch and Peters, 2010). However, investigations by confocal laser-scanning microscopy (CLSM) of living plants in microscopy rhizosphere chambers (Micro-ROCs), and by electron microscopy after freeze substitution indicated that the peripheral cytoplasmic layer in sieve tubes may contain previously unknown elements (Froelich et al., 2011). The absence of structural components from exudates has prevented biochemical and molecular studies. The alternative isolation method, extraction from homogenates, is difficult as well, since sieve tube components are attached to the plasma membrane via small protein linkers (Ehlers et al., 2000; Froelich et al., 2011). When the tissue is homogenized, these linkers lead to mixtures of different quantities of the various sieve tube components that have different densities, impeding the formation of specific bands in density gradients. The surprising exclusion of sieve element plastids from textbooks as a plastid type deriving from proplastids exemplifies the dilemma. It is comparatively easy to purify the large quantities of chloroplasts, chromoplasts, and leuoplasts that are floating in the cytoplasm of numerous cells, restricted only by transient connections to the cytoskeleton via motor proteins (Vick and Nebenführ, 2012). Isolating the small numbers of sieve element plastids that are attached rigidly to the plasma membrane is a different ball game.

The situation is less difficult for non-dispersive P-protein bodies (NDPPBs; for a review, see Behnke, 1991), which are visible in the light microscope and can be found in ~10% of the angiosperm families. At least in some cases, NDPPBs seem to move freely in the sieve tube lumen, as indicated by their preferential localization at the downstream end of the sieve element (Peters et al., 2006). Nonetheless they are absent from exudates, since their size exceeds the sieve plate pore diameter. On the other hand, their size allows them to be isolated and analysed individually. The analysis of one particular type of NDPPBs, the contractile forisomes, has not only elucidated forisome evolution (Peters et al., 2010) but also led to the molecular identification of a family of dispersive P-proteins (Pelissier et al., 2008). Starting with NDPPBs and forisomes, and proceeding to the related dispersive P-proteins, we will critically discuss current ideas about the function of these phloem components. Because we believe that there are valid alternatives to currently popular interpretations of several key experiments, we shall add some iconoclastic speculations in our final section.

Forisome function: seeing is believing—what about knowing?

Form and shape of NDPPBs vary and often are specific for certain taxa (Behnke, 1991). Some NDPPBs are capable of rapidly switching between a low-volume state at the low Ca2+ levels that are typical of transporting sieve elements, and a high-volume state at the increased Ca2+ levels of stressed or injured sieve tubes (Knoblauch et al., 2001; Pickard et al., 2006; Peters et al., 2007). This peculiar, Ca2+-dependent but ATP-independent contractility of NDPPBs is known only from the papilionoid legumes (the Fabaceae sensu stricto); in fact, it appears to be one of the synapomorphies that define this huge taxon as a monophyletic clade (Peters et al., 2010).

From principles of fluid dynamics alone, it is clear that NDPPBs must affect fluid flow in sieve tubes. Just like sieve plates and the lateral borders of the sieve elements, NDPPBs contribute to the total hydraulic resistance in the system. The contractile NDPPBs of the papilionoids, however, are unique as their shape and size, two factors that control the hydrodynamic properties of an object, change dependent on the cytosolic Ca2+ level which can be regulated by the cell (Knoblauch et al., 2001; Pickard et al., 2006; Furch et al., 2009). The active regulation of hydraulic resistance and the passive, merely structural contribution to total hydraulic resistance are fundamentally different phenomena. For these reasons, papilionoid NDPPBs were re-named gate bodies, or forisomes (Knoblauch et al., 2003). Their postulated function, however, proved hard to demonstrate in situ.

Micrographs produced by CLSM and transmission electron microscopy (TEM) of forisomes in the high-volume state in situ were highly suggestive of a structural block (Knoblauch et al., 2001). However, if based on the visual appearance of forisome plugs alone, the conclusion that forisomes actually are blocking phloem flow will remain problematic at best, for several reasons. First, what appears like a
block on a 2D picture does not necessarily block fluid flow in 3D reality, since open passages may exist outside of the 2D plane. Secondly, some materials that appear just as dense as forisome plugs on electron micrographs allow fluids to per- meate at significant rates. Cell walls, for example, look quite solid, but aqueous solutions readily pass through them; otherwise common phenomena such as plasmolysis would be inexplicable, as botanists realized more than a century ago (de Vries, 1877; Pfeffer, 1877). Apoplastic transport (i.e. fluid flow in the cell wall space) has been monitored using non-membrane-permeant dyes (Hanson et al., 1985; Moon et al., 1986). Unfortunately, the apoplastic movement of dyes does not necessarily provide a quantitative measure for concurrent water fluxes since hydrophobic wall components found, for example, in Casparian strips inhibit the apoplastic movement of water and solutes selectively (Zimmermann and Steudle, 1998). Generally, the identification of such barriers requires functional tests and cannot be achieved by simply looking at micrographs (Schreiber et al., 1999; Hose et al., 2001; Ranathunge and Schreiber, 2011). We see no reason to assume that the hydrodynamic behaviour of forisome plugs and other protein agglomerations in sieve tubes necessarily is less complex than that of cell wall materials. Thirdly, the 3D geometry of the sieve tubes containing forisomes cannot be ignored if we are to evaluate the efficiency of forisome plugs. An analysis of anatomical data available at the time indicated that forisomes were incapable of occluding sieve tubes for geometric reasons in Vicia faba (Peters et al., 2006), but the popularity of the idea that forisomes could block sieve tubes apparently remained unaffected. A causal relationship between forisome activity and phloem flow stoppage was implied by Thorpe et al. (2010) who reported that the transition of forisomes into the high-volume state correlated with the stoppage of phloem transport following rapid cooling, but, as always, correlation does not imply causation. Many plants exhibit cold-shock-inducible transient stoppages of phloem translocation (Lang and Minchin, 1986), but only papilionoid legumes possess forisomes. Therefore, the reported temporal correlation of forisome phase change and flow stoppage in a papilionoid species (Thorpe et al., 2010) does not imply a causal relationship between the two phenomena, which might well be separate effects of a common cause. On the other hand, forisomes can be isolated by pre-purifying phloem tissue before extraction and gradient centrifugation (Knoblauch et al., 2003), opening up the possibility to study their proposed function in vitro. The first published attempt to regulate fluid flow in channels on microfluidics chips using isolated forisomes failed: the movement of suspended particles, but not that of the fluid, stopped when the forisomes switched into their high-volume state (Uhlig et al., 2008). Apparently, all these problems were no match for the suggestive power of micrographs that showed occlusion of sieve elements or artificial microchannels by forisomes, and occasionally wishful thinking took over. Groscurth et al. (2012, p. 3077), for example, celebrated ‘the technological potential of forisomes, as recently demonstrated by their incorporation as smart materials into a prototype microfluidic system to control fluid flow (Uhlig et al., 2008)’. Ironically, controlling fluid flow is exactly what Uhlig and colleagues had not accomplished, as mentioned above.

As it turned out, the main problem working with isolated forisomes is that their reactivity sharply deteriorates as soon as they are released from their cells. Only after isolation procedures had been optimized, and after the incubation conditions had been re-designed to mimic closely the reducing milieu in the phloem, did it become possible actually to demonstrate the occlusion of artificial sieve elements by forisomes (Knoblauch et al., 2012). On the basis of this prima facie evidence generated by direct functional tests, it would appear most unreasonable to doubt that forisomes are capable in principle of controlling fluid flow in natural sieve tubes. However, there is to date still no direct demonstration of such flow control in vivo. Assuming that forisomes actually do occlude sieve tubes when prompted by a rise in cytosolic Ca2+, what could be a biological context in which such a reaction would be adaptive?

The plant phloem is attacked by various specialized consumers that extract phloem sap from more or less intact sieve tubes (Dixon, 1975; Douglas, 2006; Walling, 2008). Therefore, the possibility that forisomes might be involved in the defence against aphids and other phloem sap thieves is obvious (Knoblauch et al., 2001). Aphids secrete gelling saliva that hardens rapidly to form a stylet sheath as they penetrate the plant tissue with theirstylets (Miles, 1999). They also intermittently discharge watery saliva while probing as well as during phloem sap feeding (Miles, 1999; Tjallingii, 2006; Moreno et al., 2011), suggesting that watery saliva may have a dual function in target as well as non-target tissues. Watery saliva contains proteins including a variety of enzymes (Miles, 1999; Harmel et al., 2008; Carolan et al., 2009; Rao et al., 2013) and factors thought to induce or suppress plant defence responses (Hilker and Meiners, 2010; Hogenhout and Bos, 2011; Consales et al., 2012; Coppola et al., 2013; Elzinga and Jander, 2013). An essential role in phloem sap feeding has been demonstrated for Protein C002 from the pea aphid (Acrithosiphon pisum; Mutti et al., 2008). Putative calcium-binding proteins have been found in the watery saliva of a leafhopper (a non-aphid phloem feeder; Hattori et al., 2012), and in those of several aphids (Carolan et al., 2011; Nicholson et al., 2012; Rao et al., 2013). In an in vitro assay, calcium-binding proteins from the saliva of the aphid Megoura viciea competed for Ca2+ with forisomes isolated from V. faba. This interference resulted in an inhibition of the forisomes’ transition into the Ca2+-induced high-volume state (Will et al., 2007). In this experiment, protein concentrates derived from artificial media on which aphids had fed were used; it remained unexplored how the concentrations of saliva protein in these artificial concentrates compared with those that could realistically be expected to build up in functional sieve elements if delivered into the flowing sieve tube sap by an aphid. Another problem is that according to Miles (1999, p. 49), the validity of saliva analyses based on secretions into non-natural food sources is generally questionable, because of the excretory function of the glands from which the watery saliva is derived. It should be stressed also that any pair of arbitrarily chosen calcium-binding proteins...
will show competition for Ca\(^{2+}\) in tests of this type, so that observed interferences do not necessarily indicate physiological relevance. Notably, watery saliva is secreted right from the start of tissue penetration, long before a sieve tube is impaled (Moreno et al., 2011). This opens up the possibility that the physiological target of the Ca\(^{2+}\)-binding saliva proteins is not located in the phloem at all. Despite these caveats, Will and colleagues (2007) definitely have identified a candidate saliva protein that might interfere with forisome function in vivo.

Will et al. (2007) also documented a sudden shift in the electrical penetration graph (EPG) pattern of aphids feeding on Vicia leaves that occurred shortly after the leaf had been burned 5 cm from the aphid, in the upstream direction of phloem flow (Will et al., 2007). This EPG pattern shift was interpreted as a switch from phloem sap ingestion (E2 pattern) to salivation (E1 pattern) behaviour, which supposedly coincided with the plausible but undocumented stoppage of phloem flow following burning. Will et al. (2007) suggested that the aphids reacted to the postulated burning-induced sieve tube occlusion by secreting watery saliva into the sieve element in order to unplug the tube. It is worth noting that the aphid saliva could not possibly have prevented the assumed forisome-dependent stoppage of phloem flow that had been triggered by burning the leaf several centimetres upstream (source-ward) of the aphid (Will et al., 2007). Phloem transport velocities measured in intact plants ranged from 0.25 mm s\(^{-1}\) to 0.4 mm s\(^{-1}\) (Windt et al., 2006), implying that the entire contents of a large V. faba sieve element of 250 μm length (Peters et al., 2006) are completely replaced every 0.6–1 s. Thus, in an operating sieve tube, watery saliva will be strongly diluted and carried away immediately in the downstream direction, ruling out the possibility that saliva components could interact with P-proteins upstream of the inserted aphid stylet (the preferential translocation of salivary components towards sinks has been demonstrated in principle, but the temporal resolution of those experiments—24 h—did not allow for conclusions concerning fast processes on the cellular scale; Madhusudhan and Miles, 1998). Similarly, it seems practically impossible that the saliva was responsible for the assumed reopening of the phloem in the experiments of Will and colleagues. In a blocked sieve tube with stagnant contents, injected saliva components can travel by diffusion only. Therefore, it is conceivable that a significant concentration of saliva components could build up in the sieve element into which they are secreted, maybe also in the adjacent sieve elements on both sides, but certainly not all the way up to the wounded tissues several centimetres away. Thus, it is unclear how the secretion of watery saliva could provide a continuous flow of phloem sap which obviously requires certain lengths of tubes.

Based on the fact that aphids secret watery saliva while penetrating sieve tubes (Prado and Tjallingii, 1994), various authors have asserted that aphids ‘release Ca\(^{2+}\)-binding proteins in the phloem sieve cells preventing occlusion of these cells upon mechanical damage by the aphid stylets’ (Hougenhout and Bos, 2011, p. 424; compare Will et al., 2009; Hilker and Meiners, 2010). This interpretation pre-supposes that stylet insertion triggers a release of Ca\(^{2+}\) into the sieve element, an idea that seems intuitive for two reasons. First, Ca\(^{2+}\) ions are involved in numerous cellular signal transduction processes in plants including the interaction with herbivorous arthropods (Maffei et al., 2007a, b) where cellular Ca\(^{2+}\) levels rise in the immediate vicinity of bite-induced injuries (Maffei et al., 2004). It must be cautioned, though, that biting herbivores and probing aphids inflict distinct types of wounds in different kinds of cells that do not necessarily launch similar responses. The notion also seems plausible because of the assumed analogy between aphid stylets and microelectrodes, which may trigger sieve tube occlusion when inserted into a sieve element [Will et al. (2007, (2013) refer to microelectrode experiments reported by Knoblauch and van Bel (1998) to support this analogy]. However, microelectrodes actually can be inserted into sieve elements without causing damage (Knoblauch and van Bel, 1998), and electrophysiological studies of sieve elements using intracellular microelectrodes are feasible (Hafke et al., 2003; Furch et al., 2009), demonstrating that the analogy does not hold. Moreover, the general facts should be stressed that in contrast to aphid stylets, microelectrodes have not been reported to produce a protective sheath around themselves as they penetrate the tissue, do not bend around cells when their tips proceed through multiple cell layers, and have a tapering shape that causes destruction in overlying tissue when deeply embedded cells are impaled. So one may ask: what is the empirical evidence supporting the idea of a stylet insertion-induced Ca\(^{2+}\) rise in sieve elements? Astonishingly, there does not seem to be any. Quite the contrary—the first published investigation into the behaviour of Ca\(^{2+}\)-regulated phloem proteins during the initial phase of aphid attack reports that forisomes did not respond to stylet insertion even before E1 salivation started (Walker and Medina-Ortega, 2012). As a result, the authors found it ‘difficult to envision a potential role of E1 salivation immediately after sieve element penetration in preventing sieve element occlusion in the pea aphid–faba bean interaction. The possibility cannot be ruled out that E1 salivation at the onset of phloem phase [i.e. the period just after sieve element penetration] serves a function totally unrelated to phloem-sealing responses’ (Walker and Medina-Ortega, 2012, p. 333). In a subsequent in vivo study, the same authors tested the hypothesis that apparent sieve element occlusions by high-volume forisomes are removed through interactions of the forisome with the saliva an aphid secretes into the sieve element. They found no differences in the behaviour of forisomes in sieve elements with and without saliva-secreting aphids (Medina-Ortega and Walker, 2013).

One has to conclude that the idea of an involvement of forisomes in the response to phloem sap-feeding insects is not supported by the empirical data available at this time. As a consequence, the interaction of concentrated aphid saliva proteins with forisomes in vitro (Will et al., 2007) is intriguing but of unclear physiological significance.

**SEO, SEOR, and legume evolution**

As mentioned above, forisomes can be isolated in large numbers (Knoblauch et al., 2003). This facilitated the
Tagging of the gene products with green fluorescent protein (GFP) resulted in fluorescent, reactive forisomes (Pélissier et al., 2008). The gene family was named sieve element occlusion (SEO; Pélissier et al., 2008)—which was bold, as no efficient sieve element occlusion by the corresponding proteins had been demonstrated. Intriguingly, the same authors found similar genes in published sequences of non-papilionoids in which forisomes have never been reported, and these sieve element occlusion-related (SEOR) genes had a homologue in the papilionoids themselves. The gene products of both groups—SEO and SEOR—could be distinguished unambiguously on the amino acid sequence level: the papilionid SEOR protein was significantly more similar to non-papilionid SEORs than to papilionid SEOs, and both groups were defined by unique sets of conserved motifs (Pélissier et al., 2008). Taken together, these findings prompted the hypothesis that ‘a previously not characterized, well-defined group of proteins [i.e. SEO] exists in higher plants including the Fabaceae, from which the evolution of SEO proteins in the Fabaceae originated’ (Supplementary Data 3 of Pélissier et al., 2008). Supposedly, the SEO gene family had branched from the widely distributed SEOR gene family in that lineage that gave rise to the last common ancestor of the papilionoid legumes (Peters et al., 2010). The idea is in agreement with the fact that no P-proteins other than forisome-forming SEOs have been shown to respond to Ca²⁺ (for reports of unsuccessful attempts, see Knoblauch et al., 2001; Froelich et al., 2011). Available evidence thus suggests that Ca²⁺ responsiveness evolved in the ancestral protein at the root of the SEO protein family (Peters et al., 2010). It is worth emphasizing that this interpretation is in line with current views of legume evolution (for an overview, see Legume Phylogeny Working Group, 2013).

In the following year, Lin et al. (2009) detected a protein homologous to the one now called AtSEOR1 (compare Froelich et al., 2011) in the phloem proteome of Cucurbita maxima. At the same time, the cucumber (Cucumis sativus) genome was published by Huang et al. (2009), leading to the identification of a cucumber homologue of the Arabidopsis gene that encodes AtSEOR1. Huang and collaborators concluded that ‘sieve element occlusion proteins (gene cluster 4754), present in all eudicots but absent from mosses and monocots, represent a novel mechanism that evolved for sealing the sieve tube system after wounding (Pélissier et al., 2008)’ (Huang et al., 2009, p. 1280; our emphasis). In this statement, Huang and co-workers confused SEOR and SEO proteins as originally defined, and jumped to a conclusion regarding SEOR function and evolution that lacked an empirical basis, and that certainly was not supported by the reference cited. However, the presence of SEO-related genes in non-papilionoids could hardly be considered surprising from here on.

Obviously unaware of the earlier discoveries, Rüping et al. (2010, p. 1) announced the ‘unexpected occurrence’ of SEO-related genes in non-papilionoids. These authors expanded the analysis of SEO/SEOR sequence similarities, and also identified possible orthologue and paralogue relationships between SEO as well as between SEOR genes, both within and between species. Unfortunately, they ignored the sequence-based distinction between SEOR and SEO gene products although their data were in line with this interpretation. Like Huang et al. (2009) before them, they used the term ‘SEO’ in an inclusive sense that comprised SEOs and SEORs, only to define a subgroup, ‘SEO-F’, that included all proteins for which an involvement in forisome formation had been demonstrated experimentally (Rüping et al., 2010). We consider this nomenclature unnecessarily confusing because it bases the definition of groups of genes partly on sequence data and partly on the gene product’s function. On the other hand, Rüping et al. (2010) never provided a rationale for rejecting Pélassier et al.’s (2008) distinction between SEOs and SEORs. Therefore, we will retain the original definitions, first and foremost because they integrate the molecular facts into the wider evolutionary picture.

Sieve tube slime: same player shoots again!

The existence of SEO-related genes in plants not shown to generate forisomes raised questions. Are the gene products located in sieve elements? If so, what is their structure, and do they possess phloem-specific functions? Tagging of Arabidopsis (Froelich et al., 2011) and tobacco (Ernst et al., 2012) SEOR gene products with fluorescent proteins revealed meshworks of SEOR filaments within sieve tubes and dense slime agglomerations that occluded the sieve elements—or so it appeared from the micrographs. Evidently, SEOR proteins represent or are at least part of the sieve tube slime of the older literature. It has long been discussed why multiple occlusion mechanisms including callose de novo synthesis and slime plug formation by P-proteins (SEO and SEOR) appear to exist (Sabnis and Sabnis, 1995). The model plant Arabidopsis possesses two SEOR genes; both AtSEOR1 and AtSEOR2 must be present for SEO filaments and agglomerations to form (Anstead et al., 2012). Soon a debate started about the possible physiological function(s) of SEOR agglomerations that presents yet another chapter of the sieve tube slime controversy that traces its origins to the middle of the 19th century (Sabnis and Sabnis, 1995).

By 1860, light microscopy had revealed the basic structural principles of elongated phloem cells with perforated end walls (Hartig, 1837) whose function appeared to be long-distance transport (Hartig, 1860). Dense proteinaceous slime masses that consistently were found on the perforated walls which separated these sieve elements were in line with the contemporary notion that sieve tubes represented a storage and transport tissue for nitrogen-rich compounds, but not for carbohydrates (Strasburger, 1891). In those days, the translocation of sugar solutions in sieve tubes seemed unlikely for a variety of reasons, and the apparent occlusion of sieve plates by protein agglomerations was one of them. It took an outsider who struggled to establish a career, Alfred Fischer, to demonstrate that the slime masses consistently observed were artefacts caused by common but inappropriate preparation.
protocol, and that the contents of live sieve elements were more or less homogeneous and apparently fluid (Fischer, 1885). Bulk fluid flow in sieve tubes thus became a plausible concept. Before long, turgor-driven bulk translocation was discussed in textbooks (e.g. Haberlandt, 1896), ultimately leading to Münch’s (1926, 1927, 1930) presentation of the conceptual framework of an osmotically generated, pressure-driven flow that dominates current thinking about the mechanisms of phloem transport (Knoblauch and Peters, 2013).

With the advent of electron microscopy in the 1930s, investigators realized that sieve elements contained structural components that had remained invisible in the light microscope. Proteinaceous slime in the lumen of sieve elements and in sieve pores, now called P-protein, made a reappearance and created a problem for Münch’s pressure flow hypothesis. The hydraulic resistance to bulk flow offered by dense protein agglomerations in sieve pores appeared too high to be overcome by pressure gradients of plausible magnitudes. Alternative explanations for phloem translocation were developed (MacRobbie, 1971; Wardlaw, 1974; see also the four review articles by Canny, Spanner, Milburn, and Fensom in Zimmermann and Milburn, 1975) yet the Münch hypothesis still prevails as the leading hypothesis. One reason was that numerous workers in the field never stopped believing that occluded sieve plates represented preparation artefacts rather than the functional state. A number of novel preparation methods were devised, and some indeed showed open pores. However, the debate remained unresolved for decades.

The digital age provided new tools such as CLSM, which enabled the live imaging of functional sieve tubes. Important findings produced with the new tool included the direct confirmation of bulk flow in the phloem, and the visualization of the formation of P-protein agglomerations on sieve plates in response to injuries (Knoblauch and van Bel, 1998). In this context, the observation of dense SEOR agglomerations in apparently functional, uninjured Arabidopsis sieve elements mentioned above came as a surprise. What was even more surprising was the fact that the apparent sieve element occlusions by SEOR agglomerations seemed to have little effect on flow velocity, as the comparison of functional sieve tubes in roots of wild-type plants and AtSEOR1 knock-out mutants showed (Froelich et al., 2011).

**SEOR proteins: fluid dynamic effects and specific functions**

*Hydraulic effects of SEOR agglomerations in intact plants*

P-protein agglomerations in sieve tubes that appear to occlude the tube have been reported to allow the passage of fluid and dissolved macromolecules (Kempers et al., 1993) before the recent studies on AtSEOR1 agglomerations (Froelich et al., 2011). To understand the counterintuitive ineffectualness of such apparent sieve tube occlusions, Froelich et al. (2011) studied AtSEOR1 sieve tube agglomerations in the roots of intact Arabidopsis in depth. Based on sieve element structure and the ultrastructure of AtSEOR1 agglomerations, the authors evaluated the contribution of the flow resistance offered by the SEOR agglomerations to the total hydraulic resistance ($R_{total}$) in the sieve tube:

$$R_{total} = n R_{lumen} + (n - 1) R_{plate} + m R_{aggl}$$

where $R_{lumen}$ is the resistance of the lateral walls of one sieve element and $n$ is the number of sieve elements in a tube, $R_{plate}$ is the resistance of a sieve plate, and $R_{aggl}$ is the resistance of one of the $m$ SEOR agglomerations present in the tube. The authors concluded that for a typical Arabidopsis sieve tube, $n R_{lumen}$ and $(n - 1) R_{plate}$ are about equal, whereas $m R_{aggl}$ is somewhat smaller, owing to the comparatively low frequency of agglomerations (~1 per 10 sieve elements). While the value of $R_{aggl}$ can only be estimated as it depends on the porosity of the SEOR protein material which is not quantitatively known, calculations based on a range of plausible assumptions suggested that bulk flow driven by turgor pressure differentials of the expected magnitudes should be possible with the observed frequency of SEOR agglomerations (Froelich et al., 2011).

There are several important conclusions to be drawn from these findings. First, a protein agglomeration in a sieve tube does not necessarily produce total occlusion, no matter how dense and tight it may look on a micrograph. Secondly, the contribution of AtSEOR agglomerations to total flow resistance is probably smaller than that of open sieve plates and that of the tube itself. Thirdly, despite its relatively small contribution to total flow resistance, the resistance offered by AtSEOR agglomerations is a significant biophysical factor; Froelich et al. (2011) estimate $R_{aggl}$ to be ~20% of $R_{total}$. Since the volumetric flow rate, $Q$, in a sieve tube relates to its driving force given by the pressure differential, $\Delta p$, and the total hydraulic resistance, $R_{total}$, according to

$$Q = \Delta p / R_{total}$$

our conclusions imply that a plant can maintain a given flow rate under increasing numbers of SEOR agglomerations as long as its phloem loading and unloading machineries are capable of increasing $\Delta p$ commensurately. Such functional adjustment does not necessarily require complex regulation (which might be hard for the plant to accomplish anyway; Thompson and Holbrook, 2003b; Thompson, 2006). Phloem flow according to Münch’s hypothesis is driven by loading and unloading in sources and sinks, respectively; the osmotically induced $\Delta p$ is generated by the loading and unloading processes, and also links them mechanistically like a transmission belt. If $R_{total}$ along the pathway rises and loading and/or unloading continues, $\Delta p$ will increase automatically, either until a new equilibrium according to Equation (2) is established, or until the loading/unloading machinery reaches maximum capacity (this effect has been measured in vivo by Gould et al., 2004).

To appreciate fully our proposed explanation of why Froelich et al. (2011) did not detect any differences in the phloem flow velocities between wild-type plants and AtSEOR1 knock-out mutants, it is important to realize that
they made their observations in intact plants growing in the newly developed Micro-ROCs. In these miniature rhizotrons, roots remain in contact with natural soil at all times, even while being observed under the microscope. Such a nearly natural environment obviously is preferable over the artificial environment provided by the usual agar plate cultivation methods when a systemic phenomenon such as phloem translocation is studied in vivo. In the whole-plant physiology approach which Froelich and co-workers took, the plants studied were intact except for a small leaf incision for fluorescent dye loading, and did not need to be removed for experimentation from the natural soil in which they grew. There is no evidence and plausible reason why one should assume that the phloem loading and unloading machineries in these plants were not fully operational. Consequently, phloem flow proceeded at similar velocities with and without SEOR proteins.

Hydraulic effects of SEOR agglomerations in excised organs

In intact plants, flow rates in the phloem ($Q$) can be maintained as long as shifts in $R_{\text{total}}$ are balanced by changes in $\Delta p$ [see Equation (2)], which requires full functionality of the loading/unloading machineries. The latter include a potent water source—the xylem—to fuel the osmotic generation of hydrostatic pressure, especially in source organs. Therefore, the influence of $R_{\text{aggl}}$ on phloem flow might become detectable in excised organs in which the ability to modulate $\Delta p$ is impaired due to the disconnection from the physiological water source. When a petiole is cut, export of fluid from the leaf through the phloem must slow down, because the refilling of the sieve elements becomes more difficult in a leaf that is disconnected from the plant’s xylem. Consequently, we may expect to see a correlation between the rate of phloem exudation and the amount of SEOR proteins in excised leaves. Such a correlation has been demonstrated for excised leaves of tobacco (Ernst et al., 2012) and Arabidopsis (Jekat et al., 2013). In both cases, the contribution of the phloem to the total exudate secreted from the excised leaves over a period of 10 min was estimated, in both wild-type plants and genetically modified plants lacking SEORs, by measuring the amount of 10 min was estimated, in both wild-type plants and genetically modified plants lacking SEORs, by measuring the amount of β-glucose exuded in the presence and absence of modified plants lacking SEORs, by measuring the amount of β-glucose exuded in the presence and absence of 10 min was estimated, in both wild-type plants and genetically modified plants lacking SEORs, by measuring the amount of altered plants lacking SEORs, by measuring the amount of SEOR proteins in excised leaves. Such a correlation has been demonstrated for excised leaves of tobacco (Ernst et al., 2012) and Arabidopsis (Jekat et al., 2013). In both cases, the contribution of the phloem to the total exudate secreted from the excised leaves over a period of 10 min was estimated, in both wild-type plants and genetically modified plants lacking SEOR proteins, by measuring the amount of glucose exuded in the presence and absence of β-fructosidase. Under the assumption that none of the glucose but all of the sucrose present in the original exudates originated exclusively from sieve elements (for possible problems with this assumption, see van Bel and Hess, 2008; Liu et al., 2012), it was inferred that the presence of SEOR proteins reduced phloem exudation from excised leaves by factors of nine in tobacco (Ernst et al., 2012) and two in Arabidopsis (Jekat et al., 2013). It should be emphasized that SEOR proteins did not occlude (in the common sense of block) or seal the sieve tubes, but only reduced flow rate $Q$ under conditions where the capability to maintain turgor and thus $\Delta p$ in the phloem was disturbed compared with the intact plant. These findings are in full agreement with the notion that SEOR agglomerations add a summand ($m R_{\text{aggl}}$) to the total hydraulic resistance of the sieve tube ($R_{\text{total}}$), according to Equation (1). A specific wound response is not required to explain the observations.

To obtain a more intuitive picture, imagine a garden hose of 1 cm inner diameter and 1 km length; this roughly equals the length-to-diameter ratio of a sieve tube extending from the inflorescence of an Arabidopsis plant to a root tip. Clearly, one will have to apply pressure to drive water through this hose, and even more pressure will be required to drive flow through a similar hose in which solid dirt deposits increase the total hydraulic resistance by a quarter. Consequently, if we cut the clean and the dirty hose in the middle, we expect that the water will flow from the clean halves faster than from the dirty halves, and this is what Ernst et al. (2012) and Jekat et al. (2013) have shown.

The original authors seem to disagree. In the title of their paper, Jekat et al. (2013, p. 1) announced that Arabidopsis P-proteins (AtSEORs) are ‘involved in rapid sieve tube sealing’, which somewhat overstates the matter—a reduction in phloem exudation by half over 10 min. Similarly, Ernst et al. (2012, p. E1987) claimed to ‘have demonstrated clearly that P-protein accumulations block translocation following injury’. This, however, is misleading since what they showed was reduced, not blocked translocation, and because no comparison between the injured and the non-injured state was presented. Taken together, neither Ernst et al. (2012) nor Jekat et al. (2013) provided evidence to support the idea that SEOR agglomerations affect sieve tube flow through mechanisms other than a merely structural contribution to total hydraulic resistance. Their conclusion that the demonstration of such a structural contribution establishes a role for SEORs in injury responses and sieve tube sealing is logically flawed. To see why, consider viscosity, a parameter that so far we had excluded from our discussion (and from Equation 1) to keep things simple. Viscosity is the internal resistance of a medium to being sheared as in pipe flow, which the driving force of flow, in our case $\Delta p$, must overcome to initiate and maintain flow; each of the several resistive terms in $R_{\text{total}}$ [compare Equation (1)] is commonly thought to be linearly proportional to it. In transporting sieve tubes, sucrose usually is the most important solute. The viscosity of sucrose solutions depends on various physical factors, but under physiologically relevant conditions there is a straight-forward, positive relationship between sucrose concentration and viscosity (Longinotti and Corti, 2003a; Hölttä et al., 2006). However, this is analogous to what can be said about a decreased number of SEOR agglomerations, which, if all other parameters remain unchanged, will also result in an increased flow rate. To be sure, no plant physiologist will conclude that sucrose functions in sieve tube sealing following injury.

Among plant physiologists, the claim that sieve tube occlusion by P-proteins following injury prevents the loss of energetically expensive photoassimilates would hardly meet resistance. However, whether photoassimilates flowing from severed sieve tubes represent a significant contribution to injury-induced losses is not clear. In the frequently studied cucurbits, the fluid material lost from open wounds, for example after leaf excision,
specific biological processes on one hand and mere physical factors for mechanisms that may prevent sugar loss where it may be irrelevant (excised leaves) are valid models, remaining plant; whether mechanisms that apparently reduce sugar loss where it is irrelevant (excised leaves) cannot simply be extrapolated to the intact or pieces, and, even if residual phloem functionalities remain, the prevention of sugar loss following injury.

Last but not least, an obvious fact deserves to be highlighted in this context: the fitness of a plant cannot possibly increase from the reduction of sugar loss from a peripheral organ after excision of that organ. To generalize conclusions from studies on excised leaves (as done by Ernst et al., 2012; Jekat et al., 2013), one has to assume that the isolated organs are systemic phenomenon that is lost when the system is cut into pieces, and, even if residual phloem functionalities remain, not all pieces are equal after cutting. Results obtained with excised leaves cannot simply be extrapolated to the intact or remaining plant; whether mechanisms that apparently reduce sugar loss where it is irrelevant (excised leaves) are valid models for mechanisms that may prevent sugar loss where it may count (petiole stumps on the plant) is not guaranteed. **SEOR interactions with and responses to stress factors**

On the conceptual level, it is essential to distinguish between specific biological processes on one hand and mere physical necessity on the other. Forisome action provides an example of a specific biological process. Forisomes change the hydraulic resistance they offer to sieve tube flow through a process—interaction with Ca2+—that is under the control of the live sieve element, which regulates cytosolic Ca2+ in response to external stimuli. Despite the open questions discussed above, these facts very strongly support the idea that forisomes function in the regulation of phloem translocation. On the other hand, any object in the path of the flowing sieve tube contents will add to overall hydraulic resistance, tending to slow the flow. Therefore, the fact that SEOR agglomerations in sieve tubes reduce flow rates does not prove anything. It strongly suggests, though, that SEOR proteins have a beneficial function or functions that outweigh the disadvantage the plant incurs; first, by the cost of synthesis of the SEOR proteins, and; secondly, by the increased sieve tube hydraulic resistance. What do we know about potential functions of SEOR proteins in the regulation of phloem activity?

To test the responsiveness of AtSEOR agglomerations to various treatments known to induce rapid stress responses in functional sieve tubes, Froelich et al. (2011) mechanically injured sieve tubes, applied distant wounds by burning and local cold shock, and added Ca2+ to open sieve tubes and SEOR agglomerations. No immediate reactions were observed. In a few cases, the protein agglomerations started to move slowly towards the downstream sieve plate but did not occlude it; the protein rather continued its movement through the pores. This process could be followed for at least 45 min, suggesting that electron micrographs previously thought to show occluded sieve pores may in fact represent snapshots of ongoing translocation. We here report an extension of the experiments of Froelich et al. (2011). Electros shocks are known to stop phloem flow rapidly (Pickard and Minchin, 1990, 1992a, b), and we used pAtSEOR1:AtSEOR1:YFP (yellow fluorescent protein) Arabidopsis plants growing in Micro-ROCs as detailed before (Froelich et al., 2011) to investigate the possible involvement of SEOR proteins. Through small holes made in the walls of the Micro-ROCs, we placed electrodes in the soil next to a root ~1 cm apart from each other and applied voltages of 10–120 V at pulses of 1–5 s. Even at a field strength of 8 kV m⁻¹, there was no visible reaction of SEOR proteins. However, at field strengths >10 kV m⁻¹, SEOR agglomerations disappeared. However, this could hardly be considered a specific response because at the same time irreversible distortions of the entire root system occurred.

Taken together, the idea of an involvement of AtSEORs in targeted sealing mechanisms finds no support in the lack of AtSEOR responses to stimuli known to affect sieve tube transport. But do Arabidopsis plants without SEORs respond to such stimuli as their wild-type conspecifics do? We studied wild-type plants (which obviously produced SEOR proteins), pAtSEOR1:AtSEOR1:YFP transgenic plants (in which fluorescent AtSEOR1 could be observed microscopically), and SEOR knock-out plants (which lacked SEOR agglomerations). In all three plant types, phloem translocation in roots was monitored by FRAP (fluorescence recovery after photo-bleaching) after the feeding of CFDA (carboxyfluorescein hydrates exuding from excised leaves by approximately two-thirds in tobacco. This estimate is much higher than that for Arabidopsis, indicating large differences between species in the same experiment. Nonetheless, the general conclusion remains: photosynthate leakage due to continuing phloem translocation is not always the most dramatic loss a wounded plant experiences. This fact as such does not speak against a specific function for SEOR proteins in sieve tube sealing, but it questions the relative importance of any such function, should one exist, for the prevention of sugar loss following injury.

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diacetate) into the phloem, employing the methods we have described before (Froelich et al. 2011). The plants were kept in Micro-ROCs during the experiment and were cold shocked by applying ice water to the hypocotyl and most proximal part of the root system. As expected, the AtSEOR1 agglomerations visible in the pAtSEOR1:AtSEOR1:YFP plants showed no reaction. Phloem flow, however, stopped within seconds in all three plant lines (Fig. 1; Supplementary Movie S1 available at JXB online), even in SEOR knock-out lines. These results indicated that Arabidopsis does not require SEOR proteins to halt phloem translocation in response to cold shock.

It has been speculated that P-proteins block sieve pores in Arabidopsis in response to the insertion of an aphid stylet into the sieve tube (Kuśnierczyk et al., 2008). If the idea is correct and applicable to SEOR proteins, we should expect that aphids exploiting Arabidopsis greatly benefit from living on AtSEOR knock-out mutants rather than on wild-type plants. However, the opposite seems to be true: aphids exhibited decreased fitness (in terms of life time offspring production) when grown on plants lacking SEOR proteins (Anstead et al., 2012), indicating that the presence of SEOR proteins may even be beneficial to the insects at least in the compatible interaction of *Myzus persicae* and *Arabidopsis*. This finding is not totally unexpected, as amino acid availability often limits aphid growth and reproductive success (Sandström and Moran, 2001; Douglas, 2006), and aphids seem to prefer host plants growing on N-rich substrates (Nowak and Komor, 2010). Moreover, at least some aphid species possess proteolytic enzymes to break down ingested phloem proteins (Pyatti et al., 2011), an ability that previously had appeared doubtful (for a review, see Kehr, 2006). Therefore, it is not entirely implausible that SEOR proteins and their building blocks may actually increase the nutritional value of the phloem sap for aphids. Plants may benefit from the presence of P-proteins due to increased resistance in other plant–insect interactions and P-proteins may be one reason for incompatibilities. However, only direct experimental evidence can validate this idea.

In summary, currently available information suggests that (i) SEOR proteins do not seal the phloem efficiently in the case of injury; (ii) SEOR agglomerations show no visible responses to selected stimuli known to slow or halt phloem translocation; (iii) SEOR proteins show no visible responses to Ca$^{2+}$, an effector widely thought to control sieve tube occlusion; (iv) SEOR knock-out plants display qualitatively unchanged cold-shock-induced stoppage of phloem transport; and (v) SEOR proteins promote the well-being of phloem sap-feeding aphids, at least in compatible interactions of *M. persicae* and *Arabidopsis*. These findings do not support currently popular notions concerning the physiological roles of dispersive P-proteins in general and SEORs in particular. We conclude that the biological function of SEOR proteins remains obscure at this time.

**Iconoclastic speculations...**

We believe that valid interpretations of several key experiments are possible that go against the grain of currently popular notions. In this section, we present some of these iconoclastic ideas. We do not necessarily think that they are all correct; however, we do feel that the current debate might benefit from considering alternative views.

**... on forisomes**

According to a current model, forisomes occlude sieve elements in response to a transient membrane depolarization (sometimes referred to as a ‘plant action potential’) that...
originates from sites of injury (leaf burning, in particular) and travels from there along the vasculature at velocities in the range of 1 mm s⁻¹ (Furch et al., 2007). The membrane depolarization coincides with Ca²⁺ influx into the sieve element; thus, the travelling ‘plant action potential’ is accompanied by a travelling cytosolic Ca²⁺ wave. However, the rise in cytosolic Ca²⁺ brought about by the ‘action potential’ has been reported to be too weak to trigger the transition of forisomes into the high-volume state (Furch et al., 2009). Supposedly, an amplification of the Ca²⁺ signal by Ca²⁺ released from the ER is high-volume state (Furch et al., 2009). Why does flow stop in the first place? Furch and co-workers do not give details on the destruction caused by the burning injury that they applied to initiate the flow stoppage/forisome transformation cascade, but remarked that tissue movements under the microscope were inevitable consequences of the pressure waves induced by burning (Furch et al., 2009, p. 2121). It is inconceivable that the sieve tube network at the burning site survived such treatment without structural damage, implying that sieve tubes were opened, resulting in a pressure drop and a stoppage of translocation in the vicinity of the burned tissue. This forisome-independent, initial stoppage of translocation at the wounding site may have allowed forisomes to switch into the high-volume state, thereby triggering the expanding flow stoppage/forisome transformation cascade that Furch and colleagues analysed.

We speculate that under the conditions described above, forisomes might not function in stopping flow, but rather in locking an idling sieve tube network in its physiologically passive state. In this interpretation, forisomes could be viewed as analogues of the plaster cast around a broken ankle, providing stability to the system by preventing any attempts to perform normal function, thus enabling undisturbed repair activities.

…the on P-proteins and aphids

Screening the recent literature, one can hardly escape the conclusion that the role of Ca²⁺-induced sieve tube occlusion in defending the plant against attacks by phloem sap-feeding insects is firmly established (e.g. Goggin, 2007; Kuśnierscyz et al., 2008; Hilker and Meiners, 2010; Consales et al., 2011; Hogenhout and Bos, 2011; Kamphuis et al., 2013; Rodriguez and Bos, 2013; Will et al., 2013). As the references usually given in this context show, the notion rests exclusively on the finding that Ca²⁺-binding proteins from concentrated aphid saliva can inhibit the Ca²⁺-dependent transition of isolated forisomes into the high-volume state in an in vitro assay (Will et al., 2007). It is worth stressing once again that neither the prevention of sieve tube occlusion by Ca²⁺-binding saliva components nor the removal of existing occlusions by such components have been demonstrated experimentally (cf. Medina-Ortega and Walker, 2013).

Nonetheless, the results Will et al. (2007) produced with V. faba were generalized by several authors to cover angiosperms in general, despite the facts that forisomes are specific to the papilionoids and that no Ca²⁺ responsiveness has ever been reported from phloem proteins other than forisomes. For example, Kuśnierscyz et al. (2008, p. 1109) presented a model of defence mechanisms in Arabidopsis in which a ‘rising concentration of Ca²⁺ in sieve elements initializes protein clogging’. The incorrect notion that P-proteins
other than forisomes respond to Ca\textsuperscript{2+} in such a manner has been promoted by claims such as: ‘Occlusion is triggered by Ca\textsuperscript{2+} influx induced by damage (Knoblauch and van Bel, 1998)’ (cited from Will et al., 2009, p. 3305). However, while Knoblauch and van Bel (1998) certainly documented the formation of supposedly irreversible depositions of cell components on sieve plates following severe injury, they did not mention, let alone demonstrate, a role for Ca\textsuperscript{2+} in the process. Will et al. (2009) expanded their original aphid behavioural experiment (Will et al., 2007) to four plant species including three dicotyledons and Hordeum vulgare, a member of the monocotyledonous Poaceae, or grass family. They also determined the stoppage of bulk flow, and found no significant differences between the four plant species regarding flow stoppage and aphid saliva secretion as induced by leaf burning. The authors concluded that sieve plate plugging by phloem proteins is a universal phenomenon occurring in all species, even the grass H. vulgare (Will et al., 2009). However, H. vulgare lacks P-proteins (Evert, 1971) as grasses do in general (Eleftheriou, 1990). Therefore, the conclusion that the presence of P-proteins is entirely unrelated to flow stoppage and aphid behaviour in these experiments is at least equally plausible.

For argument’s sake, let us ignore the empirical evidence (Froelich et al., 2011; Walker and Medina-Ortega, 2012; Medina-Ortega and Walker, 2013) for a moment and assume that the insertion of an aphid stylet into a sieve tube triggers sieve plate occlusion by Ca\textsuperscript{2+}-responsive P-proteins. Will et al. (2007) had interpreted the switch from E2 to E1 EPG patterns after a burn stimulus as indicative of occlusions of the sieve tubes on which their aphids were feeding. Later they could induce similar EPG switches by reducing the hydrostatic pressure in an artificial feeding system (Will et al., 2008). This seemed to make good sense to Will et al. (2009, p. 3305) who maintained that ‘sieve tube occlusion is accompanied by a decrease of sieve tube pressure (Gould et al., 2004)’ (see also Will et al., 2013, p. 6). However, Gould et al. (2004) had demonstrated a decrease in turgor only downstream (sink-ward) of the sieve tube block; on the upstream (source-ward) side, turgor actually increased—exactly as we would expect if the loading/unloading machinery remained operational while the sieve tubes were blocked. On which side of a stylet insertion-induced sieve plate occlusion would we find the aphid? On the upstream or source-ward side of course, because any Ca\textsuperscript{2+} entering the sieve element at the penetration site will promptly be carried away in the downstream direction, and only there, downstream or sink-ward of the aphid, could it induce a protein plug. Thanks to the occlusion of the tube just downstream of the penetration site, the aphid would find itself at the downstream terminus of a continuous pipe connecting it directly to the source tissues. Any volume of phloem sap the aphid may remove would immediately be replaced, especially (but not only) if turgor increases.

We cannot think of any reason why the aphid would want to release a sieve tube occlusion of this kind, and therefore speculate that sieve tube occlusions are not generally a bad thing for phloem-sap thieves. Our chances of elucidating the enigmatic biological function of P-proteins would probably not suffer if the consistently reiterated dogma that aphids need to prevent sieve tube plugging in order to enjoy a continuous flow of nutrients were to be carefully re-evaluated in a fluid dynamics context.

... on phloem exudation and wound sealing

In the late 19th century, Alfred Fischer (1885) demonstrated that the slimy occlusions, which at the time were interpreted as functionally essential components of sieve elements, were in fact artefacts caused by tissue preparation and fixation for light microscopy. Fischer had discovered that by cutting through the phloem, he could induce the formation of slime agglomerations on that side of sieve plates that was facing away from the cut. He concluded that the slime had been carried to its position by the surging of the phloem sap towards the open cut, and hypothesized that the wounding-induced artefacts ‘served, so to speak, as provisional seals of the sieve tube system’ (Fischer, 1885, p. 236). More than a century later, we still have not identified possible physiological functions in the intact plant of the proteins Fischer called sieve tube slime. However, since Fischer’s artefacts consistently occur when we prepare a plant for experimentation a little too clumsily, we have come to see the biological function in the artefact, assuming or rather implying implicitly that the evolution and phylogenetic conservation of energetically costly P-proteins was and is driven only by the adaptive benefits of a protein-based emergency shut-down system that works in parallel with an already existing calllose synthesis machinery (for a review, see Eschrich, 1975; a critical view is offered by Sabnis and Sabnis, 1995). We cannot exclude that the idea is correct; but neither can we exclude that our position is analogous to that of an extraterrestrial observer who, after having witnessed a few traffic accidents from his remote vantage point, concludes that the main function of automobiles is the prevention of direct contact between fast moving humans and obstacles in their path.

The provisional seal hypothesis of P-protein function appears intuitively plausible; plants must shut down injured sieve tubes promptly to avoid losing expensive photosynthates. But is that so always? Zhang and co-workers recently suggested ‘that the role of P-proteins in the cucurbits may be to prevent excessive water loss from wounded xylem as much as it is to seal wounded phloem’ (Zhang et al., 2012, p. 1881). This suggestion is based on the observation of P-proteins that exude from severed sieve tubes rapidly in large amounts to form plugs that cover the entire cut surface of the vascular bundles. An argument following the same logics had been put forward by Read and Northcote (1983) who suggested that lectins arriving at wound sites by phloem exudation carry out an anti-invasive role. These postulated functions obviously require the exact opposite of what usually is assumed: in order to deliver functionally important substances to wound areas, sieve tubes need to remain unoccluded to enable the loss of sufficiently large amounts of P-proteins and other factors, together with expensive photoassimilates. Apparently, the assumption that plants must rapidly seal injured sieve tubes to prevent losing expensive materials is not quite as self-evidently correct; but neither can we exclude that our position is analogous to that of an extraterrestrial observer who, after having witnessed a few traffic accidents from his remote vantage point, concludes that the main function of automobiles is the prevention of direct contact between fast moving humans and obstacles in their path.
true as it sometimes sounds. At least occasionally, the phloem seems to function like lactifers and secretory ducts, the defensive tube networks present in many tracheophytes that fulfill their ecological functions by extensive secretion (Franceschi et al., 2005; Pickard, 2008; Agrawal and Konno, 2009); the extrafascicular phloem of the cucurbits may even be specialized for such a role in defense (Turgeon and Opara, 2010; Zhang et al., 2010; Gaupels and Ghirardo, 2013). In this context, we are intrigued by the following thought. When a small herbivore chews away on a leaf, why should the plant allow sieve tubes to occlude? Photoassimilates lost through severed sieve tubes at the site of biting cannot be saved by sealing the tubes as they will be lost anyway with the herbivore’s next bite; would it not seem beneficial to crank up phloem loading in the leaf to export as much photoassimilate as possible in the remaining time, rather than locking transportable goodies in a doomed organ? Plants respond differently to feeding herbivores and mechanical injury (Baldwin, 1988; Korth and Dixon, 1997; Reymond et al., 2000; Bricchi et al., 2010), so differential responses by the phloem to continuing biting as opposed to single wounding events are not implausible. However, we do not intend to speculate about herbivore–plant interactions; what we are suggesting is that plugging sieve tubes in response to injury is not obviously and always a good idea. Whether a plant benefits from injury-induced sieve tube occlusions depends on the nature of the agent that inflicted the injury, the nature of the injury, and its position. If cases could be identified in which injury-induced sieve tube plugging by P-proteins evidently harms the plant—if, in other words, sieve tube plugging could be shown to be maladaptive—a strong argument against sieve tube plugging as the primary biological function of P-proteins could be made.

Conclusions

Proteinaceous sieve tube slime, aka P-proteins, has bamboozled plant physiologists for more than a century. We think that there are two main reasons. First, the rapid reaction of some types of P-proteins to injuries makes it difficult to distinguish unambiguously between their state in the functional, transporting sieve element on one hand and preparation-induced artefacts on the other. Secondly, some of the assumed preparation-induced artefacts actually may represent the functional state of P-proteins. We came to realize that the problem is aggravated by the linguistic sloppiness in many publications including some of our own. To say that a sieve tube is occluded, sealed, clogged, or plugged is not (and should not be meant as) a statement about how the tube looks, but about its functional state. If we would use these terms only in cases in which microscopically visualized putative occlusions, seals, or plugs actually had been demonstrated to be temporally associated with stoppage of phloem translocation, the terms would become rare in our literature while we would be forced to take the fluid dynamics of the phloem seriously and analyze hydraulic resistances quantitatively. Another essential point in the elucidation of P-protein function is the apparent reversibility of any observed responses, which provides prima facie evidence for biological regulation. We consider it less than helpful when reversible processes such as forsome responses and callose deposition (Knoblauch et al., 2001; Furch et al., 2007) are compounded with the irreversible effects of catastrophic structural failure (demonstrated, for example, by Knoblauch and van Bel, 1998) into all-embracing, overly generalized hypotheses, especially when evident functional differences between taxa are ignored. This seems to be the case with some current notions about aphid–plant interactions (cf. Smith and Boyko, 2007; Cooper et al., 2011).

The direct observation of fully operational sieve tubes harboring SEOR proteins in Arabidopsis plants that were growing in an almost natural environment produced intriguing results (Froelich et al., 2011). AtSEOR agglomerations showed no visible reactions to various stimuli known to induce a slowing of phloem flow. Under certain circumstances, AtSEOR filaments and agglomerations moved slowly through sieve plates, providing an exemplary justification for our above argument: describing P-proteins visible within sieve plate pores on static micrographs as occlusions certainly is misleading, at least in Arabidopsis. Most importantly, the presence of AtSEOR proteins did not seem to inhibit phloem flow in vivo, leading Froelich et al. (2011, p. 4435) to conclude that ‘transport occurs through agglomerations’. In other words, SEOR agglomerations need not always associate with an infinite hydraulic resistance in intact plants; thus the idea of their involvement in wound sealing appears questionable.

This of course leaves us with a conundrum. If, as it now seems plausible, SEOR agglomerations do not associate with infinite hydraulic resistance in intact plants, then how are we to explain the rapid cessation of label movement down the stems of plants in response to sudden chilling, drastic intracellular pH change, audio frequency vibration, and electroshock (cf. Pickard and Minchin, 1992b)? Moreover, the chilling sensitivity is very widely distributed in the dicots (Lang and Minchin, 1986).

It can hardly be doubted that SEORs and other structural P-proteins contribute to the hydraulic resistance of sieve tubes. Their physiological functions, however, still remain elusive. We think that in vivo studies of P-protein dynamics in combination with flow velocity measurements, although methodologically demanding, represent the most promising approach to overcome this scientific roadblock, especially if methodologies can be developed that enable the monitoring of continuous sieve tubes and networks.

Supplementary data

Supplementary data are available at JXB online.

Movie S1. Cold shock experiment in a root of an AtSEOR1 knockout plant. When the ice-cold water is applied, the root moves slightly and the second, unbleached phloem file enters the plane of focus. However, refocusing occurs within a second and slowing as well as halt of phloem transport can be seen. This movie corresponds to Fig. 1.
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